

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Currently amended) A method for producing a recombinant glycoprotein in a uni- or multicellular fungal host cell which includes an  $\alpha$ -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity and is diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and which produces N-glycans comprising ~~Man<sub>5</sub>GlcNAc<sub>2</sub>-or~~ GlcNAcMan<sub>5</sub>GlcNAc<sub>2</sub> structures, the method comprising the step of expressing in the host cell a nucleic acid encoding a mannosidase enzyme that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Man $\alpha$ 1,3 and Man $\alpha$ 1,6 glycosidic linkage to the extent that at least 10% of the Man $\alpha$ 1,3 and/or Man $\alpha$ 1,6 linkages of the substrate are hydrolyzed *in vivo*, whereby expression of said mannosidase produces one or more desired N-glycan structures on a recombinant glycoprotein expressed in said host cell wherein the desired N-glycan is characterized as having at least the oligosaccharide branch Man $\alpha$ 1,3 (Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn.
2. (Currently amended) A method for producing a recombinant glycoprotein in a uni- or multicellular fungal host cell which includes an  $\alpha$ -1,2-mannosidase and a GlcNAc transferase I (GnT I) and is diminished or depleted in the activity of an initiating  $\alpha$ -1,6-mannosyltransferase and which produces N-glycans comprising ~~Man<sub>5</sub>GlcNAc<sub>2</sub>-or~~ GlcNAcMan<sub>5</sub>GlcNAc<sub>2</sub> structures, the method comprising the step of expressing in the host cell a nucleic acid encoding a mannosidase enzyme that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Man $\alpha$ 1,3 and Man $\alpha$ 1,6 glycosidic linkage, whereby expression of said chimeric mannosidase produces one or more desired N-glycan structures on a recombinant glycoprotein expressed in said host cell, wherein the desired N-glycan is produced within the host cell at a yield of at least 10 mole percent and wherein the desired N-glycan is characterized as having at least the oligosaccharide branch Man $\alpha$ 1,3 (Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn.

3. (Cancelled)

4. (Cancelled)

5. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme is capable of hydrolyzing *in vivo* both Man $\alpha$ 1,3 and Man $\alpha$ 1,6 linkages of an oligosaccharide substrate comprising a Man $\alpha$ 1,3 and Man $\alpha$ 1,6 glycosidic linkage.

6. (Original) The method of claim 1 or 2, wherein the oligosaccharide substrate is characterized as Man $\alpha$ 1,3 (Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; GlcNAc $\beta$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; GlcNAc $\beta$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; GlcNAc $\beta$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; Man $\alpha$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; Man $\alpha$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; Man $\alpha$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn or high mannan.

7. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme is a Class 2 mannosidase enzyme.

8. (Previously presented) The method of claim 7, wherein the Class 2 mannosidase enzyme has a substrate specificity for GlcNAc $\beta$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; GlcNAc $\beta$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; or GlcNAc $\beta$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn.

9. (Previously presented) The method of claim 7, wherein the Class 2 mannosidase enzyme is one which is normally found in the Golgi apparatus of a higher eukaryotic host cell.

10. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme comprises a Class IIx mannosidase activity.

11. (Previously presented) The method of claim 10, wherein the Class II $\alpha$  mannosidase enzyme has a substrate specificity for Man $\alpha$ 1,3 (Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; or Man $\alpha$ 1,2 Man $\alpha$ 1,3 (Man $\alpha$ 1,3 Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn.

12. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme comprises a Class III mannosidase activity.

13. (Previously presented) The method of claim 12, wherein the Class III mannosidase enzyme has a substrate specificity for (Man $\alpha$ 1,6 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; (Man $\alpha$ 1,3 Man $\alpha$ 1,6) Man $\beta$ 1,4-GlcNAc  $\beta$ 1,4-GlcNAc-Asn; or high mannans.

14. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme is overexpressed.

15. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme is further capable of hydrolyzing a Man $\alpha$ 1,2 linkage.

16. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme has a pH optimum of from about 5.0 to about 8.0.

17. (Canceled)

18. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme is localized within the secretory pathway of the host cell.

19. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme is localized within at least one of the ER, Golgi apparatus or the trans Golgi network of the host cell.

20. (Previously presented) The method of claim 1 or 2, wherein the nucleic acid encoding the mannosidase enzyme encodes an enzyme comprising a mannosidase catalytic domain fused to a targeting peptide.

21. (Previously presented) The method of claim 20, wherein the mannosidase catalytic domain is native to the host cell.

22. (Previously presented) The method of claim 20, wherein the mannosidase catalytic domain is heterologous to the host cell.

23. (Previously presented) The method of claim 1 or 2, wherein the mannosidase enzyme is selected from the group consisting of *Arabidopsis thaliana* Mannosidase II, *C. elegans* Mannosidase II, *Ciona intestinalis* mannosidase II, *Drosophila* mannosidase II, Human mannosidase II, Mouse mannosidase II, Rat mannosidase II, Human mannosidase IIx, Insect cell mannosidase III, Human lysosomal mannosidase II and Human cytoplasmic mannosidase II.

24. (Previously presented) The method of claim 20, wherein the targeting peptide is native to the host cell.

25. (Previously presented) The method of claim 20, wherein the targeting peptide is heterologous to the mannosidase catalytic domain.

26. (Original) The method of claim 1 or 2, further comprising the step of isolating the glycoprotein from the host cell.

27. (Original) The method of claim 1 or 2, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, *Fusarium gramineum*, *Fusarium venenatum* and *Neurospora crassa*.

28. (Original) The method of claim 27, wherein the host cell is *Pichia pastoris*.

29. (Original) The method of claim 1 or 2, wherein the glycoprotein is a therapeutic protein.

30. (Original) The method of claim 29, wherein the therapeutic protein is selected from the group consisting of erythropoietin, cytokines, coagulation factors, soluble IgE receptor  $\alpha$ -chain, IgG, IgG fragments, IgM, interleukins, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin,  $\alpha$ -1-antitrypsin and  $\alpha$  - feto protein.

31 – 56. (Cancelled)

57. (New) The method of claim 1, wherein the desired N-glycan comprises an oligosaccharide structure selected from the group consisting of Man<sub>3</sub>GlcNAc<sub>2</sub>, GlcNAcMan<sub>3</sub>GlcNAc<sub>2</sub>, and Man<sub>4</sub>GlcNAc<sub>2</sub>.

58. (New) The method of claim 2, wherein the desired N-glycan comprises an oligosaccharide structure selected from the group consisting of Man<sub>3</sub>GlcNAc<sub>2</sub>, GlcNAcMan<sub>3</sub>GlcNAc<sub>2</sub>, and Man<sub>4</sub>GlcNAc<sub>2</sub>.